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Assessment of Foliar Biochemicals in Tropical, Subtropical and Temperate Ecosystems of Lesser Himalayas

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ABSTRACT

Biophysicochemical characteristics of forests influence the exchange of energy and material among organisms, and between cryosphere and biospheres. Present study analyses foliar nitrogen and lignin of dominant plant species and its distribution pattern across tropical, subtropical and temperate forests in Lesser Himalayas in Doon Valley based on 26 field samples plots of 0.1 ha using CHNS elemental analyser. Potentiality of foliar chemical assessment has also been investigated using Continuum removed spectral absorbance and band depth analysis of Analytical Spectral Device (ASD) based foliar spectral responses. The nitrogen and lignin concentrations varied from 9.70 to 35.50 mg g^{-1} and 215.00 to 435.00 mg g^{-1}, respectively. Herbaceous species have more foliar nitrogen than tree species on weight/unit area basis. The species present in tropical ecosystem showed higher foliar nitrogen than temperate species. The mean foliar nitrogen of the tropical, subtropical and temperate species varied between 11.00 mg g^{-1} to 35.5 mg g^{-1}, 11.2 mg g^{-1} to 32.9 mg g^{-1} and 9.7 mg g^{-1} to 17.7 mg g^{-1}, respectively. The foliar lignin content is inversely related to the foliar nitrogen content in all cases. ANOVA analysis was insignificant along altitudinal gradient due to minor differences. Continuum removed spectral absorbance (CRSA) and band depth analyses of spectral responses for three dominant species from each ecosystem showed strong absorption/response by foliar nitrogen content in the spectral regions 536nm, 546nm, 560nm, 638nm, 680nm and 810nm and therefore, indicate potential for foliar nitrogen estimation and as an alternative to laboratory analyses.

Key Words: Foliar Biochemical Characteristics; Lignin; Nitrogen; Band Depth Analyses.

INTRODUCTION

Vegetation has major influence on the exchange of energy and material between the atmosphere and the Earth's surface due to its biophysical and biochemical characteristics (Bacour et al. 2002). Plant biochemical composition, specifically lignin and nitrogen contents are linked to several aspects of biogeochemical processes such as stress monitoring, soil carbon balance, forest health and productivity (Melillo et al. 1982, Brik and Vitousek 1986, Field and Mooney 1986, Aber and Federer 1992, Tripathi and Singh 1992, Wessman 1994, Berg 2000, Ghoshal and Singh 2010). Plant species contain quite similar biochemical constituents but these vary in the proportions. Even plants of the same species may vary in the proportions of their biochemical contents (Li 2004). The variation in the biochemical proportions in the vegetation is a function of species trait, seasonal variability, environmental variability, age composition, light interaction and rate of photosynthesis (Field 1983, Evans 1989, Bonan 2008). Species trait, nitrogen availability and climate influence the foliar nitrogen content. However, foliar content of lignin is largely controlled by the environmental adaptations. The lignin is also considered as the response material of the disease attack on vegetation as a result of its defence mechanisms (Nicholson and Hammerschmidt 1992, Wu et al. 1997, Eisa M. et al. 2013, Pan and Saddle 2013, Tripathi and Agrawal 2013). Lignin, lignin: nutrient ratios and nitrogen have been identified as key characteristics determining the rate of organic matter turnover, decomposition rate of leaf litter and extra-cellular enzymatic activity in forests (Tripathi and Singh 1992, Jina et al. 2003, Sinsabaugh et al. 2005, Heim and Schmidt 2006, Keeler et al. 2009, Ghoshal and Singh 2010). Thus the information related to the biochemical composition of different forest type canopy is valuable in planning and management of ecosystem and their role in ecological services. Therefore, the mapping and monitoring of the vegetation biochemicals are vital for understanding productivity and physiology in changing environmental conditions. It could also be valuable for the spatially distributed modeling of vegetation productivity, nutrient cycling, vegetation stress, evapotranspiration, surface energy balance (Curran et al. 1992, Wessman 1994, Turner et al. 1999) and to determine the functioning of the ecosystems. Present study was taken up to estimate the foliar chemicals specially nitrogen and lignin of dominant plant species across tropical, subtropical and temperate regions of the lesser Himalayas in Doon Valley. It is hypothesised that there would be variation in the leaf biochemical proportion of the plant species of different forest types at different elevations and leaf-age. The study would help in the geospatial modelling and mapping of canopy biochemicals and in understanding ecosystem processes. This communication reports pattern of nitrogen and lignin distribution of 31 dominant and co-dominant species.

The classical Kjeldahl method is most commonly used for determining nitrogen in food, feed, fertilizer and plant material which involves digestion, large amount of reagents, laboratory setup and time (Kjeldahl 1883). However, Nelson and Sommers (1973) developed an efficient method by placing plant tissue samples in pyrex Folin-Wu tubes and digested it with a salt-catalyst-sulphuric acid mixture by heating the tubes in an aluminium block. Further, Hach et al. (1985) used peroxymono-sulphuric acid as digestive reagent for the improved nitrogen determination. Tripathi and Singh (1992) used Heraeus CHN-O-S Rapid Auto–analyser for the determination of C and N in bamboo leaves in presence of Acetanilide (C_8H_9NO) standard. Penga et al. (1995) used SPDA-502 chlorophyllmeter estimates to estimate nitrogen in correlation with the leaf area and specific leaf weight and reported correlation coefficient (r) = 0.81for leaf area based nitrogen estimate and r= 0.43 for dry weight basis nitrogen estimate. Spectroscopic method using spectral response correlation has been used to estimate leaf nitrogen content at 2100 nm wavelength (Curran et al. 2001, Kokaly 2001). Narrow-band reflectance ratios for the estimation of nitrogen at leaf and canopy level have been attempted. The changes in leaf N were best correlated with either R_{695} nm or R_{755} nm in leaves and either R_{410} nm or R_{700} nm in canopies and reported r^2= 0.70 for narrow waveband ratio (R_{415}/R_{710}) and nitrogen (Read et al. 2002). Xue et al. (2004) applied canopy spectral reflectance to monitor leaf nitrogen status in rice and found ratio index of NIR to green (R_{810}/R_{560}) as useful ratio index. Zhao et al. (2005) worked on the selection of optimum reflectance ratios for estimating leaf Nitrogen. Kokaly et al. (2009) have reviewed all the aspects of spectroscopy in relation to the non-pigment material viz., lignin, nitrogen, water and cellulose. Vyash et al. (2012) developed algorithms for estimating biochemical in plant of tropics using allometry and spectroscopy.

The methods for estimating lignin in vegetation samples are based on two basic principles: (a) gravimetric methods (van Soest 1963, Quarmby and Allen 1989) and (b) spectrophotometric methods (Wessman et al. 1988, McLellan et al. 1991, Joffre et al. 1992, Gillon et al. 1993, Bolster et al. 1996, Curran et al. 2001, Kokaly et al. 2009). Kögel (1986) worked on estimation and decomposition pattern of the lignin component in forest humus layers by using oxidative degradation of material with CuO. Jacquemoud et al. (1996) estimated leaf biochemistry including lignin also using the PROSPECT leaf optical properties model. Martin and Arber (1997) developed calibration equations, relating lignin and nitrogen with first-difference spectral bands with R^2 =0.77 and 0.87, respectively. Brinkmann et al. (2002) compared three most commonly applied method of lignin estimation in leaves and litter: (a) thioglycolic acid (TGA), (b) acetylbromide (AB) and (c) acid detergent fiber (ADF) method. Takahashia et al. (2004) applied near Infra-red spectroscopy for lignin estimation to compare the two types of lignin, i.e. acid detergent and acetyl bromide in fallen leaves. Quantification of lignin content using standard gravimetric approach is accurate but time consuming and costly (Mthembu

2006). Morrison (2006) used semi-micro method for the determination of lignin in forage crops to predict its digestibility. Zhai et al. (2013) studied nitrogen, phosphorus and potassium content in the leaves of different species using laboratory spectroscopy and compared partial least-square regression (PLSR) and support vector machine regression (SVMR) methods and concluded that the SVMR is better method for the estimation of biochemical components. A very few attempts have made to assess lignin in natural forests in India. In the present study two ecologically important phytochemicals, i.e. nitrogen and lignin in the foliage of tropical, subtropical and temperate forests occurring in Doon valley in Lesser Himalayas have been attempted and standard wet chemistry gravimetric method (TAPPI) has been used for the estimation of leaf lignin.

STUDY AREA

The study area lies in Doon Valley-Mussoorie Hills in the Lesser Himalayas of Uttarakhand state (Figure 1). It spans from tropics of Siwalik Hills to the temperate Mussoorie hills and is located between 77° 59' to 78° 05' E longitude and 30° 26' to 30° 08' N latitude. It covers an area of about 114.00 km^2, which is a forested landscape dominated by *Shorea robusta* Gaertn. f. (Sal) in tropical Sal forest and Oaks (*Quercus* spp.) in temperate Oak forest and mixed tropical and subtropical forests, agriculture and settlement in between. In between Oak and Sal is subtropical Pine forest.

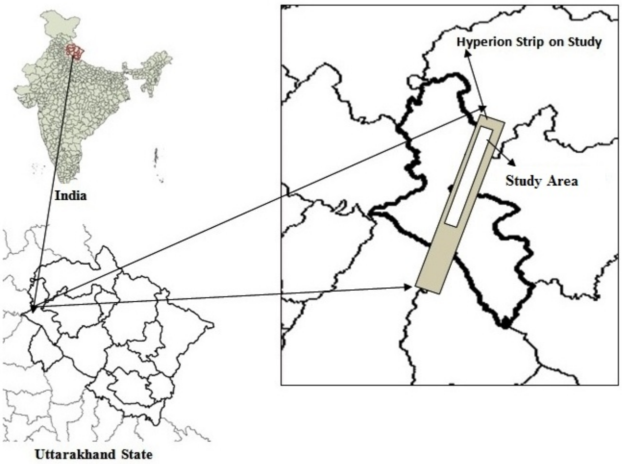

Figure 1. Location of the study area

METHODOLOGY

Field Data Collection

The classified map of species/communities matrixes based on Hyperion EO-1 hyperspectral data (Row/Path 146/39) of December 25, 2006 was used for sampling design and it covered three elevation ranges (±100 m), i.e. 600 to 1000m, 1000 to 1700m and more than 1700m. Analytical Spectra Device (ASD) and Global Positioning System (GPS) Trimble Juno ST were used for collection of tree species spectra and location, respectively. Carbon-Hydrogen-Nitrogen-Sulphur (CHNS) elemental analyzer available at IIRS and Soxhlet Extraction Apparatus available at FRI, Dehradun were used for foliar chemical analysis. For classification, field-based spectra collected using ASD were applied. Using stratified random sampling approach 26 sample plots of 0.1 ha each were laid for collection of field data on trees, shrubs and herbaceous layers. Five leaf-samples from each of 31 species were collected before the onset of winter (September and early October in 2010) soon after end of monsoon. Species leaf spectra were also collected for different species, age, locations, etc. ASD Spectroradiometer (range from 350-2500 nm) collected species spectra data in 1251 bands at 2 nm band widths. Earlier, the ASD device was calibrated for the 'Dark Current Correction' and then with the "Spectralon" white reference panel for the generation of the reference spectra. After calibration the Force Optics Probe gun (input device) of the device was directed vertically on the leaf of the plants to collect spectral signature. About 20 spectra of each plant sample were taken and averaged out for band depth analysis so that the influence of atmospheric effect and error due to sampling could be minimised. The same leaves were collected as sample to estimate their biochemical composition. Further the collected sample of the plant leaves were immediately packed into the poly-bag and tagged properly. This was done to avoid moisture loss of the leaf sample during its transport from field to laboratory. After taking fresh weight of whole leaf the oven dried leaf samples at 70°C in laboratory were weighed again and used to estimate the water content of the leaves. Leaf samples of the same plant were taken for the analysis of nitrogen content in laboratory and then for the correlation analysis with band depth, to check the applicability of electromagnetic energy absorption in modelling and mapping of nitrogen at canopy and landscape level using remote sensing and geo-information science in three different climatic zones.

Laboratory Analysis of Foliar Biochemicals

Estimation of Nitrogen

Nitrogen content was estimated using CHNS elemental analyser. It works on the principle of the 'Dumas method' which involves complete and instantaneous oxidation of the sample by 'flash combustion'. Chromatographic column in the analyser separates the combustion products such as NO_2, CO_2, SO_2 and H_2O. The thermal conductivity detector (TCD) present in CHNS analyser detects these combustion products. This gives an output signal proportional to the concentration of the individual components of the mixture. For this the leaf samples were oven dried at 70ºC for 24 hours, grinded well and passed through a 0.2mm sieve for making the particle size uniform and compatible for oxidation in CHNS elemental analyser. The ground samples were injected into CHNS elemental analyser using aluminium boxes. The digital reading of the percentage foliar N was noted down.

Estimation of Foliar Lignin

Lgnin content was estimated using standard Tappis method (Schoening and Johansson1965). It had two steps: (a) hydrolysation and solubilisation of carbohydrate in leaf by sulphuric acid, and (b) filtering, drying and weighing of acid insoluble lignin. The leaves were air dried. The leaf samples were extracted with 95% alcohol for 4 hours in Soxhlet apparatus. The extractive free dust of the sample was then hydrolysed with 15ml 72% H_2SO_4 for 2 hrs. The hydrolysed products were then transferred to a conical flask containing 560ml distilled water, and was heated at 70°C for 4 hrs. After cooling, it was filtered through a previously weighed G3 crucible. The crucible containing lignin was placed in an oven overnight and cooled. The weight of lignin with crucible was estimated, and from that the total lignin percentage in the sample was estimated.

Spectral Analysis of Foliar Nitrogen

Spectral signatures collected from field were processed and resampled in 10nm band width to be able to correlate these spectral signatures with the Hyperion EO-1 imagery. Further the continuum removal was applied over these resampled spectra to minimise illumination effect and to enhance differences in absorption (Clark and Roush 1984). Continuum removed spectral absorbance (CRSA) analyses of spectral region 450 to 1450nm for dominant tropical (*Shorea robusta* Gaertn. f.) and temperate species (*Quercus leucotrichophora* A. Camus) were made using formula, 1-continuum removed spectral reflectance. Band depth analysis was done on continuum-removed spectra between 545 and 720nm which comes under the spectral ranges used by Mutanga and Skidmore (2004a b and c) and further by Sykioti et al. (2011) for vegetation biophysicochemical content estimation. For calculating band depth following formulae were used:

$$BD = 1 - R' \quad \text{(Mutanga and Skidmore 2004c)} \qquad (1)$$

where, BD is Band Depth and R' is the continuum-removed spectra which have been obtained using following equation

$$R' = R/Rc \quad \text{(Mutanga and Skidmore 2004c)} \qquad (2)$$

where, R is the reflectance value at each point of absorption pit and R_c is the reflectance level of the continuum line at the corresponding absorption pit.

The continuum-removed spectrum minimizes the influence of factors such as water absorptions, atmospheric anomalies, soil background, and BRDF effects (Kokaly and Clark 1999). So the band depth calculated from continuum-removed spectrum would be able to provide better estimates of foliar biochemical. Band depth values of corresponding species reflectance have been correlated with their average percentage of the foliar nitrogen. Dominant species from three different climatic systems such as tropical (*Shorea robusta* Gaertn. f.), subtropical (*Pinus roxburghii* Sarg.) and temperate (*Quercus leucotrichophora* A. Camus) were selected for the band depth analysis.

RESULTS AND DISCUSSION

Foliar Nitrogen

The foliar nitrogen of 31 species is given in Table 1. Foliar nitrogen of the *Acacia catechu* L.f (riverine species) is the highest (35.5 mg g^{-1}) among all the species, while *Cryptomeria japonica* (L.f.) D. Don has lowest foliar nitrogen (9.7 mg g^{-1}). Compound-leaf of *Acacia catechu* L.f) with less venation is the main reason behind elevated nitrogen content (Bojović and Marković 2009). Intra-species foliar nitrogen variability (for mature leaves) shows higher nitrogen content in younger plants than the older plants (Table 1) on unit area/weight basis. Leathery thicker leaves (*Ficus benghalensis* L.) have less nitrogen (15.8 mg g^{-1}) than in thin leaves of young *Shorea robusta* Gaertn. f. (16.4 mg g^{-1}) under the same climatic condition (tropical). The mean foliar nitrogen of the temperate species varies between 9.7 mg g^{-1} to 17.7 mg g^{-1} except *Eupatorium* sp. and *Grewia optiva* where both these species showed unexpectedly high mean foliar nitrogen content (27.8 and 33.1 mg g^{-1}). *Grewia optiva* is the most frequently utilised species in the area (as fodder, making rope, fuel wood, etc.). Higher nitrogen content of the *Grewia optiva* indicates its high potential as fuel for forest fire. Compound leaves of the tropical species *Murraya koenigii* (L.) Sprengel (an associate of the *Shorea robusta* Gaertn. f.) are also showing high foliar nitrogen content (32.5 mg g^{-1}, second highest content in tropical climate).

Table 1. Species-wise mean foliar nitrogen and lignin content (mg g^{-1}) along with their family and climatic regimes.

Species	Age	Habit	Climate	Family	Nitrogen	Lignin
Acacia catechu L.f	Old	Tree	Tropical	Fabaceae	35.5	360
*Anogeissus latifolia (*Roxb. ex DC.*)* Wall. ex Guill and Perr.	Old	Tree	Subtropical	Combretaceae	17.7	435
Bauhinia variegata L.	Young	Tree	Subtropical	Fabaceae	25.5	255
Bauhinia variegata L.	Old	Tree	Subtropical	Fabaceae	20.0	385
Castanea sp.	Old	Tree	Tropical	Fagaceae	17.6	355
Cedrus deodara Roxb.	Old	Tree	Temperate	Pinaceae	10.0	365
Cedrus deodara Roxb.	Young	Tree	Temperate	Pinaceae	9.9	355
Cotoneaster frigidus Wall. ex Lindl.	Old	Shrub	Subtropical	Rosaceae	11.2	380
Cryptomeria japonica (L.f.) D. Don	Old	Tree	Temperate	Cupressaceae	9.7	350
Dalbergia sissoo Roxb. ex DC.	Old	Tree	Tropical	Fabaceae	26.6	235
Dendrocalamus strictus (Roxb.) Nees	Old	Woody herb	Tropical	Poaceae	24.6	375
Eupatorium sp.	Old	Herb	Temperate	Asteraceae	27.8	240
Ficus benghalensis L.	Old	Tree	Tropical	Moraceae	15.8	405
Ficus religiosa L.	Old	Tree	Tropical	Moraceae	20.3	215
Ginkgo biloba L.	Old	Tree	Temperate	Ginkgoaceae	17.7	355
Grevillea robusta A Cunn.	Old	Tree	Temperate	Proteaceae	14.2	390
Grewia optiva J R Drumm. ex Burret	Old	Tree	Temperate	Tiliaceae	33.1	335
Impatiens sulcata Wall.	Old	Herb	Subtropical	Balsaminaceae	26.8	395
Juglans regia L.	Old	Tree	Subtropical	Juglandaceae	16.4	265
Lantana camara L.	Old	Shrub	Tropical	Verbenaceae	11.9	270
Mangifera indica L.	Old	Tree	Tropical	Anacardiaceae	16.9	330
Murraya koenigii(L.) Sprengel	Old	Shrub	Tropical	Rutaceae	32.5	325
Pinus roxburghii Sarge	Old	Tree	Subtropical	Pinaceae	13.0	350
Pinus wallichiana A.B. Jackson	Old	Tree	Subtropical	Pinaceae	12.2	365
Platanus orientalis L.	Old	Herb	Subtropical	Platanaceae	21.4	245
Pongamia pinnata (L.) Pierre	Old	Tree	Subtropical	Fabaceae	32.9	345
Quercus leucotrichophora A. Camus	Middle	Tree	Temperate	Fagaceae	16.9	375
Quercus leucotrichophora A. Camus	Old	Tree	Temperate	Fagaceae	14.6	395
Quercus leucotrichophora A. Camus	Young	Tree	Temperate	Fagaceae	14.6	235
Rhododendron arboreum Smith	Old	Tree	Temperate	Ericaceae	11.0	335
Shorea robusta Gaertn. f.	Old	Tree	Tropical	Dipterocarpaceae	14.2	390
Shorea robusta Gaertn. f.	Young	Tree	Tropical	Dipterocarpaceae	16.4	330
Syzygium cuminii (L.) Skeels	Old	Tree	Tropical	Myrtaceae	11.0	380
Tectona grandis L.f.	Old	Tree	Tropical	Verbenaceae	21.6	325
Tectona grandis L.f.	Young	Tree	Tropical	Verbenaceae	23.2	325
Thuja orientalis L.f.	Middle	Tree	Subtropical	Cupressaceae	12.6	390
Thuja orientalis L.f.	Old	Tree	Subtropical	Cupressaceae	11.2	410
Thuja orientalis L.f.	Young	Tree	Subtropical	Cupressaceae	13.5	395
Zanthoxylum armatum DC.	Old	Shrub	Subtropical	Rutaceae	25.7	335

It has been observed that the litter with less nitrogen and high lignin content (i.e. high Lignin: N ratio) degrades slowly and is little bit resistant to the microbial action (Jacob et al. 2010). Litter produced by such species which is non-native and have high foliar Lignin: N ratio will decompose very slowly as compared to the native species produced litter (with low or average Lignin: N ratio) even in the same climatic condition and ecosystem. This may disturb the balanced nutrient cycling due to following reasons, (a) accumulation of available nutrient pool by the invasive alien in their phytomass, (b) generation of low quality litter which may or may not resistant to the microbial action (c) retarded, inhibited or restricted growth of native species due to allelopathic effect of invasive alien which may result into the loss of natural nutrient accumulator or sink in particular ecosystem by the near future. As *Lantana camara* L. (invasive alien) is having less nitrogen and high lignin content i.e., high Lignin: N ratio in the foliage it is indicating its threats to the biogeochemical cycling of tropical ecosystem which may exceed up to the subtropical and temperate ecosystem in near future due to climate change. *Syzygium cumini* (L.) Skeels has thick leaves in comparison to the *Shorea robusta* Gaertn. f. is showing less nitrogen content in the same climatic conditions. Young age *Tectona grandis* L.f. has higher nitrogen content than old age *Tectona grandis* L.f.. *Pongamia pinnata* (L.) Pierre has the highest and *Cotoneaster frigidus* Wall. ex Lindl. has the lowest foliar nitrogen content in the subtropical climate, *Grewia optiva* J R Drumm. ex Burret has highest and *Cryptomeria japonica* (L.f.) D. Don has lowest foliar nitrogen content in the temperate climate and *Acacia catechu* L.f has highest and *Syzygium cumini* (L.) Skeels has lowest foliar nitrogen content (Table 1).

Foliar nitrogen in different species is categorised according to their habit, phenology, cotyledons, phylum, leaf shape, and altitudinal ecosystem distribution and is showing a specific reducing and increasing trends (Figure 2a-2f) supporting Han et al. (2005) for Chinese plants, but contrary in Indian plants by Raghubanshi (2008). The findings of Raghubanshi could be correct in term of total turnover. *Acacia catechu* L.f in riverine forest on fresh alluvial soils has the highest nitrogen content (35.50 mg g^{-1}) while the dominant temperate species *Quercus leuco-trichophora* A. Camus and *Cedrus deodara* Roxb. have lowest nitrogen content (14.60 mg g^{-1} and 9.90 mg g^{-1}, respectively).

These differences could be due to the climatic conditions, age of the vegetation, morphological structure, stand height and light availability. The riverine forests are distributed at lower elevation and dominated generally by *Acacia catechu* L.f with low height and seem to have better opportunity to assimilate for newly formed leaves the nitrogen accumulated from its retranslocation during leaf senescence (Raghubanshi 2008). Detritus accumu-lation due to surface runoff from the higher elevation could be another reason for elevated nitrogen concentration in the soil of lower altitude which may be easily available for the vegetation occurs in lower regions. At the same time *Cedrus deodara* Roxb. in temperate vegetation occurring at higher elevation (2000-3200 m) with the height of 40 to 50 m has less nitrogen content. Dominant tropical vegetation *Shorea robusta* Gaertn. f. showed intermediate nitrogen content (14.20 mg g^{-1}) occurring on old alluvial soils with respect to the temperate and riverine vegetation.

However, lower storey plants in all the vegetation types indicated irregular trends of foliar nitrogen content. At the same time herbs from different ecosystem were not showing much variation but these have more nitrogen content than their upper storey vegetation (Figure 2a). The foliar nitrogen content according to the habit of the species showed highest foliar nitrogen in herbs and lowest in the trees with intermediate in shrubs. Based on phenology categorization of different species the nitrogen content is showing decreasing trend from annuals < deciduous < evergreen < semi-evergreen (Figure 2b). Mean nitrogen content was significantly higher in dicotyledonous plant species in comparison to the monocotyledons (Figure 2c). The plant phylum-based classification, the higher nitrogen content was observed in the angiosperms and lower in the gymnosperm (Figure 2d). Analyses based on leaf-morphology and structure shows that the broad leaved species are having more nitrogen than narrow-leaved conifers (Figure 2e). Some leaf characteristic and related nitrogen variability as observed from the analyses is given in Table 2.

Altitudinal Variation in Foliar Nitrogen

It is also observed that climatic conditions along the gradient play an important role in distribution of foliar N content. The nitrogen concentration along the altitudinal gradient showed increasing trend i.e., the lower altitude (tropical ecosystem) plants have more nitrogen as compared to the plants of higher altitudinal ranges (temperate ecosystem) (Figure 2f), however, ANOVA analysis has rejected the hypothesis of nitrogen increase along the altitudinal gradient (accepted null hypothesis),

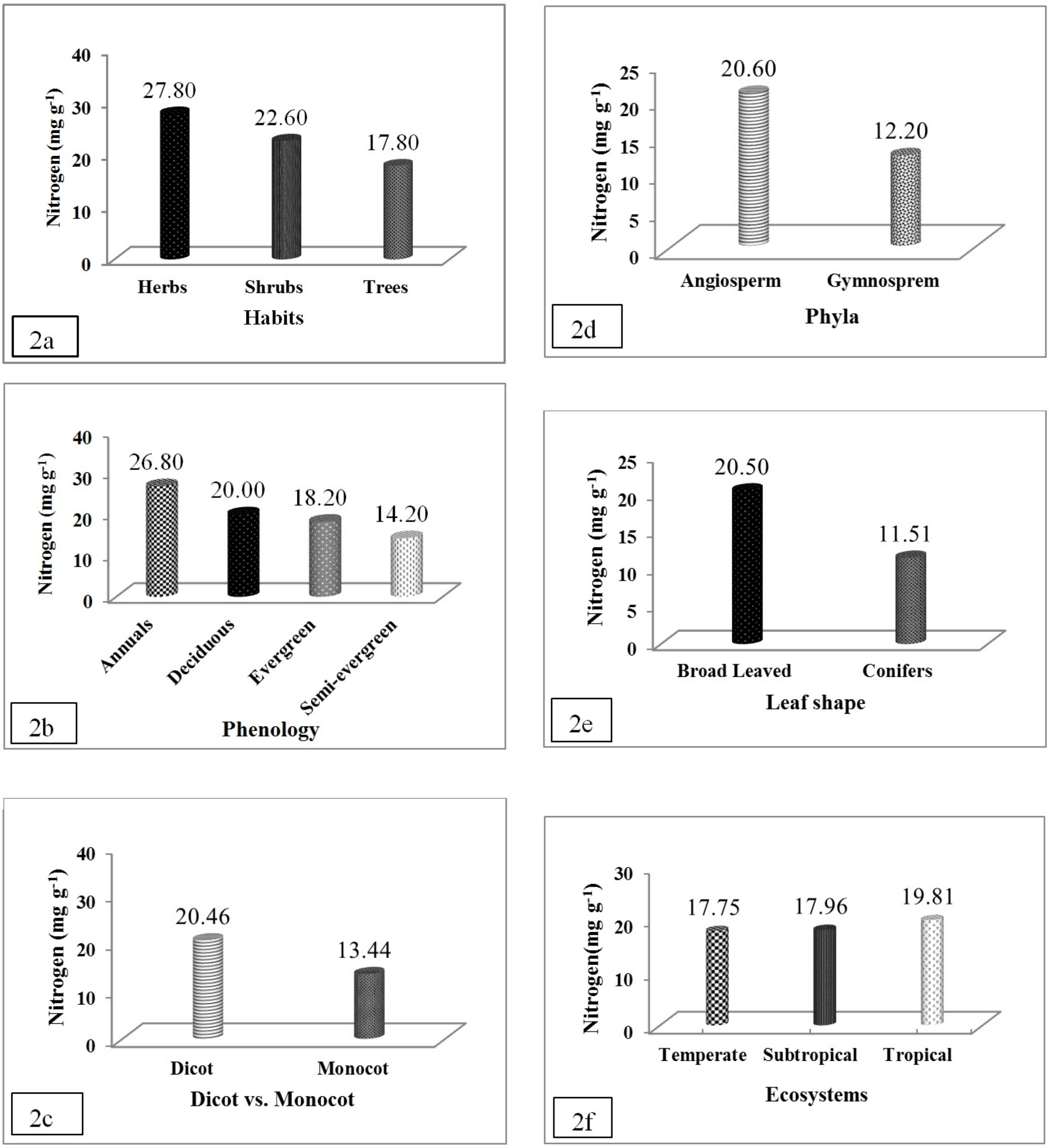

Figure 2. Mean nitrogen content (mg g^{-1} of sample).

(2a) Ddifferent life forms of vegetation; (2b) Different phenological groups; (2c) Dicotyledonous vs. monocotyledonous plants; (2d) Angiosperms vs. gymnosperms; (2e) Bbroadleaf vs narrow-leaf species; and (2f) Different ecosystems along the altitudinal gradient.

Table 2. Leaf characteristics along with nitrogen conditions as observed from analyses.

S. N.	Leaf characteristics	Nitrogen Variability
1	Thin vs. Thick	Thin leaves have more foliar nitrogen than thick leaves.
2	Compound vs. Simple	Compound leaves have more nitrogen than simple leaves.
3	Smooth vs. Hairy	Smooth leaves have more nitrogen than hairy leaves.
4	Lower surface non-coated vs. coated	Non-coated leaves have more nitrogen than coated leaves.
5	Mesophytic vs. Xerophytic	Mesophytic leaves have more nitrogen than Xerophytic (except hot desert xerophytes with compound leaves)
6	Diffused vs. Clearly visible vascular tissue in leaf venation	Leaves with diffused vascular tissue have more nitrogen than leaves with complex and clear-cut vascular tissue.

may be due to minor differences. Subtropical ecosystem shows intermediate nitrogen content. This is due to the more light intensity (open habitat) in the tropical ecosystem and less in temperate ecosystem followed by intermediate in subtropical ecosystem (Niinemets et al. 2002, Niinemets 2003).

Mean foliar nitrogen concentration indicates inverse influence of altitudinal gradient (Figure 2f) as it decreases with the increasing altitude. The tropical and lower subtropical plants (400 to 1200 m) show highest mean value (19.800 mg g^{-1}, n=9) of the foliar nitrogen concentration. Upper-subtropical zone plants (1200 to 1700 m) have intermediate mean content (18.000 mg g^{-1}, n=9) of foliar nitrogen and the temperate region plants (>1700 m) had the lowest mean value (17.75 mg g^{-1}, n=9). *Syzygium cumini, Shorea robusta, Ficus benghalensis, Mangifera indica, Ficus religiosa, Tectona grandis*, *Dalbergia sissoo* and *Acacia catechu* were considered for the lower region and the nitrogen concentration varied from 11.00 mg g^{-1} to 35.50 ±7.43 mg g^{-1}. In *Pinus wallichiana* , *Pinus roxburghii, Thuja orientalis*, *Anogeissus latifolia* and *Bauhinia variegata* from subtropical region, nitrogen varied from 12.20 to 25.50 mg g^{-1}. *Cedrus deodara*, *Rhododendron arboreum*, *Grevillea robusta* , *Quercus leucotrichophora*, *Ginkgo biloba* and *Grewia optiva* in temperate region had 9.90 to 33.10 mg g^{-1} nitrogen. All the plants studied above are woody and showed an altitudinal gradient in the distribution of nitrogen. Although this trend was not uniform for the herbaceous plants in the same vegetation and ANOVA analysis rejected the hypothesis, the results are in strong agreement with Han et al. (2005) for vegetation life forms (Table 3). It is found that the herbs have high leaf nitrogen content as compared to the woody vegetation (Pate and Dell 1984, Foulds 1993, Aerts 1996, Thompson et al. 1997, Güsewell and Koerselman 2002).

Continuum Removed Spectral Absorbance and Depth Analysis for Nitrogen variability

Continuum removed spectral absorbance (CRSA) analysis of spectral region 450 to 1450nm reveals an interesting fact. The difference in the percent nitrogen content was almost similar to the difference in CRSA values in the blue and green spectral region (400-600nm) for dominant tropical (*Shorea robusta*) and temperate species (*Quercus leuco-trichophora*). Total difference in Continuum removed spectral absorbance percentage of tropical and temperate species was 0.21 which is almost

Table 3. ANOVA analysis of Nitrogen variation along altitudinal gradient

Source of Variation	*SS*	*df*	*MS*	$F_{0.05}$	*P-value*	*F crit*
Between Groups	0.010444	2	0.005222	1.249088	0.307216	3.4668
Within Groups	0.087794	21	0.004181			
Total	0.098238	23				

similar to the percentage difference (0.20) of their foliar nitrogen content (Figure 3a). The analyses of spectral response pattern of dominant species one each from climatic region showed different band depth for nitrogen content (Figure 3b). It is seen that the band depth increases with the increase in N content in the spectral region 560nm and 638nm similar as obtained by (Mutanga and Skidmore 2004c) and by Read et al. (2002) for the spectral region 560nm. The lower tropical vegetation of *Shorea robusta* Gaertn. f., temperate vegetation of *Quercus leucotrichophora* A. Camus and subtropical vegetation of *Pinus roxburghii* Sarg. having nitrogen content 1.64%, 1.46% and 1.22% (which showed absorption/response in the bands range 546nm 560nm, 638nm and 668nm). As *Shorea robusta* is a moist deciduous species it is showing higher nitrogen content than the evergreen *Quercus leucotrichophora* (Han et al. 2005) due to high assimilation of easily available nitrogen (Raghubanshi 2008) in response to the leaf senescence. *Pinus roxburghii* Sarg. is justifying the absorption hypothesis (more material more absorption) given by Mutanga and Skidmore (2004c).

More samples and respective spectral responses are required to justify the elevation hypothesis of nitrogen content. Band depth analysis of electromagnetic energy responses (Hyperspectral remote sensing) from foliar surface is showing potential in mapping and modelling nitrogen at foliar, canopy and ecosystem level with high accuracy, efficiency and in non-destructive manner. It is also beneficial in controlling the chemical pollution of wet chemistry labs (unconsidered point source of the environmental pollution).

Assessment of Foliar Lignin

It is observed that in most of the species the foliar lignin increases with leaf-age in the same species, such as *Bauhinia variegata* L. young leaves had lignin content of 255.00 mg g^{-1} which is significantly lower than the old leaves (385 mg g^{-1}). Similar patterns were found in leaves of *Cedrus deodara* Roxb. (355.00 mg g^{-1} and 365.00 mg g^{-1}), *Quercus leucotrichophora* A. Camus (235.00 mg g^{-1} and 395.00 mg g^{-1}), *Shorea robusta* Gaertn. f. (330.00 mg g^{-1} and 390.00 mg g^{-1}) and *Thuja orientalis* L.f. (395.00 mg g^{-1} and 410.00 mg g^{-1}) for young and old leaves, respectively. It indicates that with the increase in age, leaves accumulate secondary metabolites such as lignin which helps in defence mechanisms of the leaves against outer antigens and disease causing organisms. *Anogeissus latifolia* (Roxb. ex DC.) Wall. ex Guill and Perr. has the highest (435 mg g^{-1}) and *Ficus religiosa* L. the lowest foliar lignin content (215 mg g^{-1}). However, we did not note this pattern in *Tectona grandis* L.f., and needs further investigations.

The distribution of mean foliar lignin content (per unit dry weight) among tropical, subtropical and temperate species is given in Table 2 and Figure 4 (a-f). Based on physiognomy or phenology of the vegetation, lignin shows a decreasing trend from evergreen (344.78 mg g^{-1}) > semi-evergreen (333.00 mg g^{-1}) > deciduous forests (332.05 mg g^{-1}); however these differences are insignificant. The content of foliar lignin was high in the monocotyledons (375.00 mg g^{-1}) as compared to the dicotcotyledons (330.69 mg g^{-1}). The mean foliar lignin in conifers was 372.50 mg g^{-1} whereas in broad-leaved it was less (333.00 mg g^{-1}). Gymnosperm species have more lignin in leaves (371.00 mg g^{-1}) in comparison to the angiospermic species (332.00 mg g^{-1}). Based on life form (habit) of the vegetation lignin content decreased from trees (347.57 mg g^{-1}) to shrubs (318.00 mg g^{-1}) and herbs (240.00 mg g^{-1}). Lignin content did not indicate altitudinal variation as indicated in foliar nitrogen (i.e. tropical>subtropical>temperate forests). However, foliar lignin content was high at higher altitude (>1200 m). Mean foliar lignin in tropical forest was recorded 330.00 mg g^{-1}, in temperate forest (337.97 mg g^{-1}) and highest in subtropical forests (353.57 mg g^{-1}). The distribution of lignin among all the species showed a mean value of 341.01 and standard deviation of 55.54. Our findings support those of Maithani et al. (1998) who reported inverse relation between foliar nitrogen and lignin.

CONCLUSIONS

The analysis of foliar nitrogen in the tropical, subtropical and temperate ecosystem indicates that the nitrogen is showing decreasing trend in the content along the elevation gradient, however, the ANOVA analysis rejected this hypothesis. Mean content of the nitrogen was highest in the tropical vegetation at lower elevation, intermediate in the subtropical vegetation and lowest in the temperate vegetation. It is also found that nitrogen content varies in different habits. Similarly physiognomy/ phenology also plays a significant role in accumulation of nitrogen. Broad leaved species have higher nitrogen content than coniferous. Dicotyledonous species have more nitrogen than monocotyledonous species. Similar trend has been found between angiosperms vs. gymnosperms. Analysing foliar lignin of

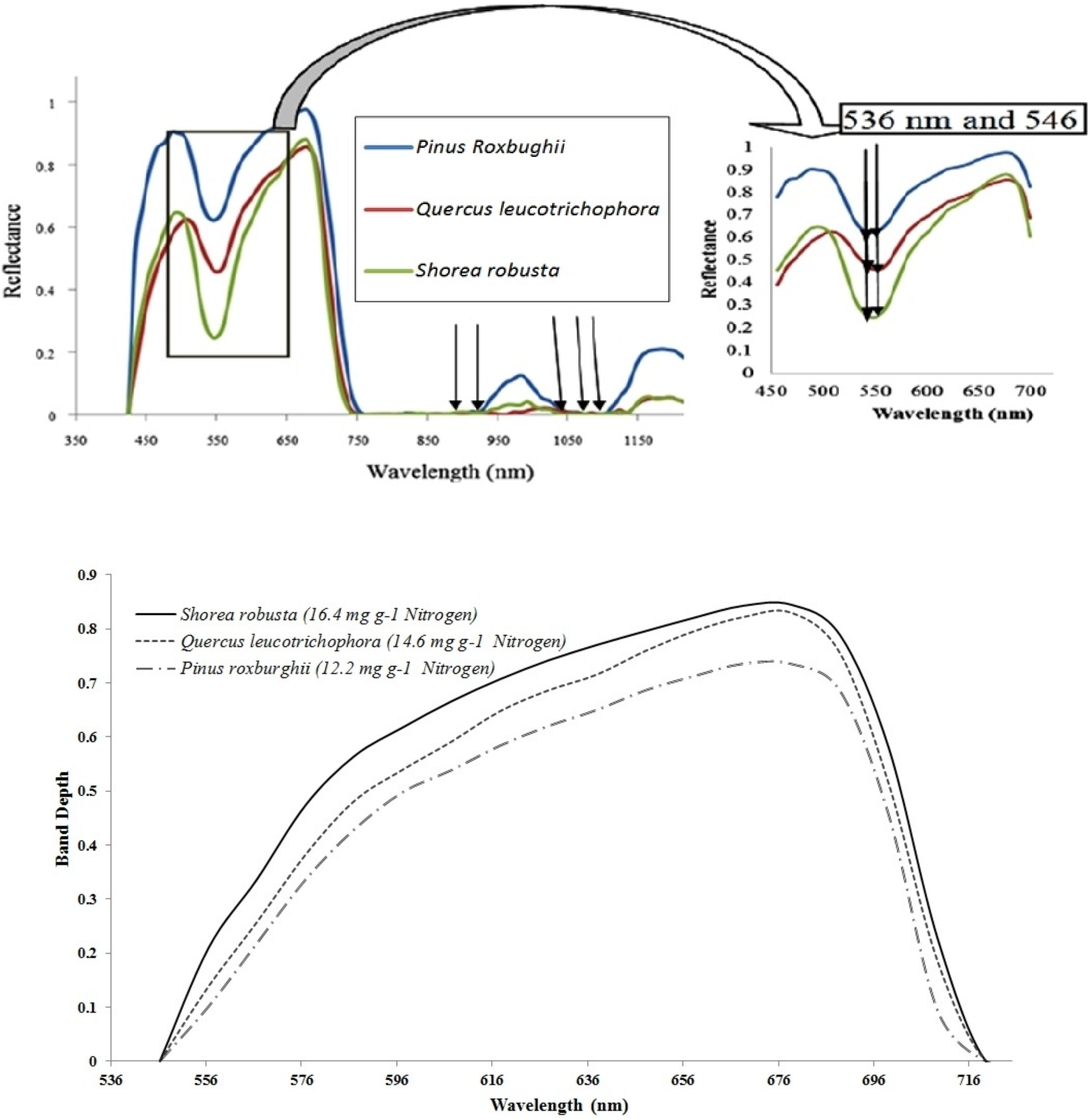

Figure (3a-b): Spectral analysis for nitrogen content estimation. (a) Continuum removed spectral absorbance (CRSA) of spectral region 450 to 1450 nm. (b) Band depth (BD) of three dominant species from three different climatic zones (25/09/2010). The band depth is increasing with the increase in the foliar nitrogen content

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dominant trees indicated lignin in decreasing order: subtropical>temperate>tropical. Phenological observations showed decreasing trend of lignin as: evergreen> semi-evergreen> deciduous. There is inverse relation between nitrogen and lignin in habit of the vegetation. Dicotyledonous plants have lower lignin content in comparison to the monocots. Similarly angio-sperms have less foliar lignin as comparison to the gymnosperm. Broad leaved vegetation have less foliar lignin content than conifers. Habit-based observation indicated decreasing foliar lignin: Trees > Shrubs > Herbs. High foliar lignin:N ratio of *Lantana camara* (invasive alien)

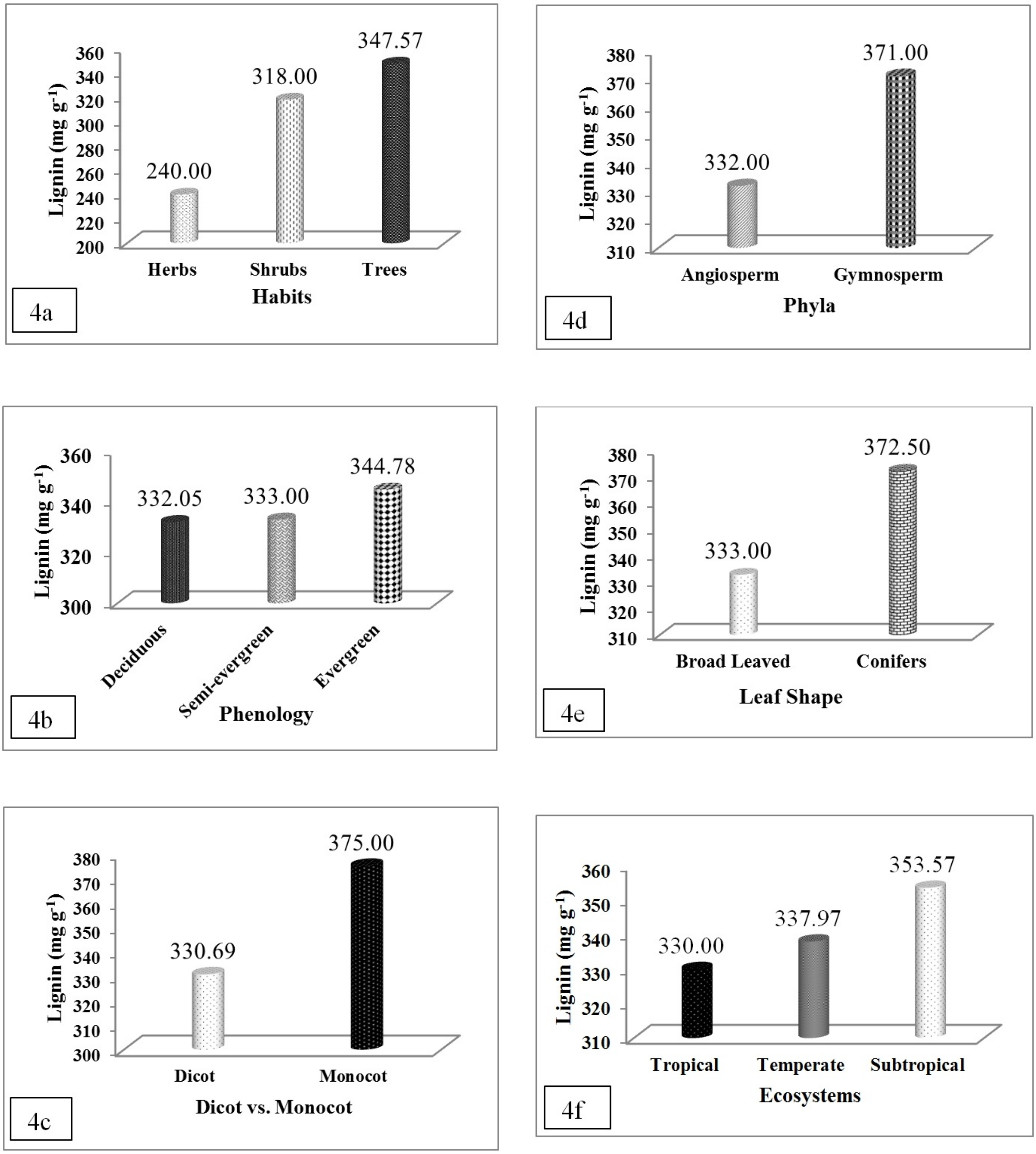

Figure (4a-f): Mean of Lignin content is given in mg g^{-1} of sample. (4a) Lignin content of vegetation differed in life form or habit of the vegetation, (4b) Lignin content in different phenological group of the vegetation,. (4c) Lignin content of different species of Dicotyledonous vs. Monocotyledonous, (4d) Lignin content based of Phylum of vegetation in Angiosperms and gymnosperms, (4e) Lignin content based leaf morphology, (4f) Lignin content of different ecosystem distributed along the altitudinal gradient.

shows potential threat to the biogeochemical cycling in tropical to lower subtropical ecosystem. Band depth analysis justifies the variation of nitrogen content along the elevation gradient but more data are required.

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